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| <p>(54) Title: USE OF IMINO SUGARS FOR ANTI-TUMOR THERAPY</p> <p>(57) Abstract</p> <p>The invention relates to methods for inhibiting the growth of tumors or other neoplasms, treating the symptoms that might be a consequence of such tumors or other neoplasms. It relates particularly to the formulation and/or administration of an effective amount of at least one of imino sugars or pharmaceutical acceptable salts thereof, 1-deoxynojirimicin (DNJ) or derivatives thereof, and glycosidase inhibitors or pharmaceutically acceptable salts thereof useful in such methods.</p> | | |

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USE OF IMINO SUGARS FOR ANTI-TUMOR THERAPY

FIELD OF THE INVENTION

The invention relates to methods for inhibiting the growth of tumors or other neoplasms, or treating the symptoms that might be a consequence of such tumors or other neoplasms. It relates particularly to the formulation and/or administration of an effective amount of a pharmaceutical compound according to the invention.

BACKGROUND OF THE INVENTION

Antitumor therapy now involves an attack on the development of malignant tumor tissue by disrupting normal metabolic processes on which the new tumor depends for growth.

Tumor growth, like the growth of normal tissues, requires the synthesis of certain cell surface glycoproteins and glycolipids. Intracellular oligosaccharide processing depends on glycosidases and glycosyl transferases that can modify the structure and composition of these glycoproteins and glycolipids. It has been known for some time that glucosidase and glycolipid synthesis inhibitors, *e.g.*, the glucose analog N-butyl-1,5-deoxy-1,5-imino-D-glucitol (N-butyl DNJ), alter the synthesis of complex oligosaccharides. By altering these structures in endothelial cells with a related inhibitor castanospermine, it was possible to inhibit tumor growth (Pili *et al.*, *Cancer Res* 33:2920-2925, 1995; Radin, *Biochem Pharmacol* 15:589-595, 1999). Use of N-butyl-1,5-deoxy-1,5-imino-D-glucitol (N-butyl DNJ) to inhibit *in vitro* and *in vivo* growth of EPEN and CT-2A brain tumors was published after the filing date of our priority documents (Ranes *et al.*, *Proc Am Soc Cancer Res* 41:258, 2000).

Many of the existing drugs, however, are poorly tolerated by individuals such that the ratio of minimum dose with therapeutic effect to maximum dose that can be safely given is low. Moreover, it can be difficult to achieve a therapeutic concentration of these drugs in some regions of the body (*e.g.*, brain cancers). There is a need for more effective drugs to treat tumors and other neoplasia, especially to inhibit the growth thereof.

Other advantages of the invention are discussed below or would be apparent to a person skilled in the art of cancer prevention and treatment from that discussion.

SUMMARY OF THE INVENTION

An objective of the invention is to provide to provide imino sugars and derivatives thereof that are effective in the treatment of tumors and other neoplastic growths.

One embodiment of the invention is treatment of a tumor or other neoplasm with an
5 imino sugar or pharmaceutically acceptable salt thereof. The amount of imino sugar or pharmaceutically acceptable salt thereof that is administered to an individual in need of such treatment is effective to slow and/or reduce growth of the tumor or other neoplasm in comparison to not treating the disease.

Another embodiment of the invention is treatment of a tumor or other neoplasm with
10 1-deoxynojirimycin (DNJ) or a derivative thereof. The amount of DNJ or derivative thereof that is administered to an individual in need of such treatment is effective to slow and/or reduce growth of the tumor or other neoplasm in comparison to not treating the disease.

A further embodiment of the invention is treatment of a tumor or other neoplastic growth (*i.e.*, a neoplasm) with a glycosidase inhibitor or pharmaceutically acceptable salt
15 thereof. The amount of glycosidase inhibitor or pharmaceutically acceptable salt thereof that is administered to an individual in need of such treatment is effective to slow and/or reduce growth of the tumor or other neoplasm in comparison to not treating the disease.

Compounds of the invention may be used to produce a medicament or other pharmaceutical composition to treat a tumor or other neoplasm.

20 In particular, a long chain N-alkyl derivative of DNJ (*e.g.*, between five and 16 carbons, inclusive, in length) is preferred for use in the invention. More preferred are long chain N-alkyl derivatives between eight and 16 carbons, inclusive, in length. N-nonyl-1,5-deoxy-1,5-imino-D-glucitol (N-nonyl DNJ) is a preferred derivative. Short chain N-alkyl derivatives of DNJ (*i.e.*, four carbons or less in length) may be used, but are not preferred.

25 Treatment with a compound that has superior bioavailability and does not lower blood glucose levels is preferred, but not necessary, to achieve the objective of the invention.

BRIEF DESCRIPTION OF THE DRAWING

Figure 1 shows the inhibiting effect of N-alkyl imino sugars N-butyl DNJ and N-
30 nonyl DNJ on experimental brain tumor growth in mice. Squares are control, diamonds are N-nonyl DNJ at 50 mg/kg/day, circles are N-nonyl DNJ at 500 mg/kg/day, and triangles are N-butyl DNJ at 500 mg/kg/day (*n.b.*, diamond and circle symbols are close to each other).

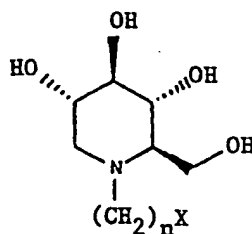
DETAILED DESCRIPTION OF THE INVENTION

In general, a tumor or other neoplasm may be treated with an imino sugar or pharmaceutically acceptable salt thereof. The amount of imino sugar or pharmaceutically acceptable salt thereof that is administered to an individual in need of such treatment is effective to slow and/or reduce growth of the tumor or other neoplasm in comparison to not treating the cancer.

The imino sugar may be a galactoside analog. Preferably, the imino sugar has an N-alkyl chain of at least five carbons and, more preferably, the imino sugar contains at least one C₅-C₁₆ substituent. Bioavailability of the imino sugar across said individual's blood-brain barrier is substantially better than N-butyl DNJ. For example, the imino sugar may be N-nonyl DNJ.

The tumor or other neoplasm may be treated with 1-deoxynojirimycin (DNJ) or a derivative thereof. The amount of DNJ or derivative thereof that is administered to an individual in need of such treatment is effective to slow and/or reduce growth of the tumor or other neoplasm in comparison to not treating the cancer. Preferably, the derivative has an N-alkyl chain of at least five carbons and, more preferably, the derivative contains at least one C₅-C₁₆ substituent. Bioavailability of the derivative across said individual's blood-brain barrier is substantially better than N-butyl DNJ. For example, the derivative may be N-nonyl DNJ.

The tumor or other neoplasm may also be treated with an effective amount of a glycosidase inhibitor or pharmaceutically acceptable salt thereof. The amount of glycosidase inhibitor or pharmaceutically acceptable salt thereof that is administered to an individual in need of such treatment is effective to slow and/or reduce growth of the tumor or other neoplasm in comparison to not treating the cancer. The glycosidase inhibitor may be 1-deoxynojirimycin (DNJ) or a derivative thereof according to Formula A,



A

wherein X may be an unsaturated straight aliphatic hydrocarbon, saturated and unsaturated branched aliphatic hydrocarbon, aromatic hydrocarbon or substituted derivatives thereof, cyclic hydrocarbon or substituted derivatives thereof, -O-Y, -S-Y, -Y-OH, -Y-NH₂, -Y-COOH, -Y-CON⁺-R, or -Y-COO-R; wherein Y may be a saturated or unsaturated hydro-

carbon, that can be a straight aliphatic hydrocarbon, branched aliphatic hydrocarbon, aromatic hydrocarbon or substituted derivatives thereof, or cyclic hydrocarbon or substituted derivatives thereof; wherein R may be hydrogen, or a saturated or unsaturated hydrocarbon that can be a straight aliphatic hydrocarbon, branched aliphatic hydrocarbon, aromatic hydrocarbon or substituted derivatives thereof, or cyclic hydrocarbon or substituted derivatives thereof; and wherein n may be a whole number less than or equal to 16. Bioavailability of the glycosidase inhibitor across said individual's blood-brain barrier is substantially better than N-butyl DNJ. For example, the glycosidase inhibitor may be N-nonyl DNJ.

A long chain N-alkyl derivative of DNJ (*e.g.*, between five and 16 carbons, inclusive, in length) may be used as the compound of the present invention. More preferred are long chain N-alkyl derivatives between eight and 16 carbons, inclusive, in length. By comparative example, the unexpected advantage of using N-nonyl DNJ (*i.e.*, the N-alkyl derivative of DNJ with a nine carbon chain) instead of N-butyl DNJ (*i.e.*, the N-alkyl derivative of DNJ with a four carbon chain) to treat a brain tumor is demonstrated. Thus, short chain N-alkyl derivatives of DNJ (*i.e.*, four carbons or less in length) may be used but are not preferred.

The compound can be administered to an individual affected by cancer, especially a solid tumor or other neoplasm. Anti-tumor activity is not necessarily related to other functions of the compound. Thus, while certain short chain N-alkyl derivatives of imino sugars (*e.g.*, N-butyl DNJ) are potent inhibitors of the N-linked oligosaccharide processing enzymes, such as α -glucosidase I and α -glucosidase II (Saunier *et al.*, *J Biol Chem* 257: 14155-14161, 1982; Elbein, *Ann Rev Biochem* 56:497-534, 1987), some compounds of the present invention may exhibit substantially little or no inhibition of a glycosidase, especially in comparison with N-butyl DNJ.

Amino and imino compounds used as starting materials in the preparation of long chain N-alkylated compounds are commercially available (Sigma, St. Louis, Missouri, US; Cambridge Research Biochemicals, Norwich, Cheshire, UK; Toronto Research Chemicals, Ontario, CA) or can be prepared by known synthetic methods. Long chain N-alkylated compounds can be prepared by reductive alkylation of amino or imino compounds. For example, the amino or imino compound can be exposed to long chain aldehyde and reducing agent (*e.g.*, sodium cyanoborohydride) to N-alkylate the amine. In particular, the compound can be a long chain N-alkylated imino sugar. The imino sugar can be, for example, deoxynorjirimycin (DNJ) or derivatives, enantiomers, or stereoisomers thereof. The compound can be prepared stereospecifically using a stereospecific amino or imino compound as a starting material. Alternatively, the compound can be purified out of a mixture of products after syn-

thesis. The compounds can be purified, for example, by crystallization or chromatographic methods.

The synthesis of a variety of imino sugars are also known in the art. For example, methods of synthesizing DNJ derivatives are known and are described, for example, in U.S. Patent Nos. 5,622,972, 5,200,523, 5,043,273, 4,994,572, 4,246,345, 4,266,025, 4,405,714, and 4,806,650, and U.S. patent application 07/851,818. Methods of synthesizing other imino sugar derivatives are known and are described, for example, in U.S. Patent Nos. 4,861,892, 4,894,388, 4,910,310, 4,996,329, 5,011,929, 5,013,842, 5,017,704, 5,580,884, 5,286,877, and 5,100,797. The substituents on the imino sugar can influence the efficacy of the compound as an anti-tumor agent and, additionally, can preferentially target the molecule to one organ rather than another.

The compounds can include protecting groups. Various protecting groups are well known. In general, the species of protecting group is not critical, provided that it is stable to the conditions of any subsequent reaction(s) on other positions of the compound and can be removed at the appropriate point without adversely affecting the remainder of the molecule. In addition, a protecting group may be substituted for another after substantive synthetic transformations are complete. Where a compound differs from a compound disclosed herein only in that one or more protecting groups of the disclosed compound has been substituted with a different protecting group, that compound is within the present invention. Further examples and conditions are found in *Protective Groups in Organic Chemistry* by T.W. Greene, 1st ed., 1981; Greene and Wuts, 2nd ed., 1991).

Compounds of the present invention may be used to produce a medicament or other pharmaceutical composition to treat a tumor or other neoplasm.

Compounds described herein may be used in the free amine form or in a pharmaceutically acceptable salt form. Pharmaceutical salts and methods for preparing salt forms are provided in Berge *et al.*, *J Pharm Sci* 66:1-18, 1977. Pharmaceutically acceptable salts can be preferred for compounds that are difficult to solubilize in the pharmaceutical composition (e.g., compounds having longer alkyl chains). A salt form is illustrated, for example, by the HCl salt of an amino derivative. For example, the compounds can be di- or tetra- acetates, propionates, butyrates, or isobutyrate. The compound can be a solvate.

The compounds may also be used in the form of prodrugs such as, for example, the 6-phosphorylated DNJ derivatives described in U.S. Patent Nos. 5,043,273 and 5,103,008.

Use of compositions which further comprise a pharmaceutically acceptable carrier and compositions which further comprise components useful for delivering the composition

to an individual are known in the art. Addition of such carriers and other components to the composition of the present invention is well within the level of skill in this art.

Pharmaceutical compositions that are useful in the present invention may be administered as an oral, ophthalmic, suppository, aerosol, topical, or other formulation. For example, it may be in the physical form of a solid, powder, tablet or lozenge, capsule, liquid or solution, gel, emulsion, suspension, syrup, or the like. In addition to the compound, such compositions may contain pharmaceutically-acceptable carriers and other ingredients known to facilitate administration and/or enhance uptake (*e.g.*, saline, dimethyl sulfoxide). Other formulations, such as nanoparticles, liposomes, and immunologically-based systems may also be used in accordance with the present invention. For a solid tumor or other neoplasm, the composition may be incorporated in a permeable matrix (*e.g.*, a bead or disk) placed adjacent to the tumor or other neoplasm for sustained, local release.

Pharmaceutical compositions may be administered by any known route. By way of example, the composition may be administered by a mucosal, pulmonary, topical, or other localized or systemic route (*e.g.*, enteral and parenteral). The term "parenteral" includes subcutaneous, intradermal, intramuscular, intravenous, intraarterial, intrathecal, and other injection or infusion techniques, without limitation.

These compositions may be administered according to the present invention in a single dose or in multiple doses which are administered at different times. Because the effect of the composition upon a tumor or other neoplasm may persist, the dosing regimen may be adjusted such that chemotherapy is promoted while the individual is otherwise minimally effected. By way of example, an individual may be administered a dose of the composition once per day, whereby growth of the tumor or other neoplasm is slowed or reduced for the entire or most of the day, while the individual's normal functions are inhibited for only a short period during the day.

Suitable choices in formulation, administration, and dosing can be made with the goals of achieving a favorable response in the individual with respect to the tumor or other neoplasm (*i.e.*, efficacy), and avoiding undue toxicity or other harm (*i.e.*, safety) thereto.

Compound of the present invention, or a pharmaceutical composition thereof, is administered to an individual in an amount effective to slow or reduce growth of a tumor or other neoplasm. The term "slow or reduce" refers to the detectable slowing of the time rate of change in size and/or reduction (*i.e.*, change in size) of growth of a tumor or other neoplasm. Tumor volume is understood to be a measure of size. The term "effective amount" refers to that amount of compound thereof necessary to achieve a therapeutic effect.

The term "treatment" refers to reducing or alleviating symptoms in an individual, preventing symptoms from worsening or progressing, and/or preventing disease in an individual who is free therefrom as well as slowing or reducing growth of a tumor or other neoplasm. For a given individual, improvement in a symptom, its worsening, regression, or progression may be determined by any objective or subjective measure. Efficacy of the treatment may be measured as an improvement in morbidity or mortality (*e.g.*, lengthening of survival curve for a selected population). Treatment may also involve debulking the tumor or other neoplasm by surgical and/or radiation therapy, especially if performed prior to chemotherapy. Thus, combination therapy with one or more medical/surgical procedures and one or more other chemotherapeutic agents may be practiced with present invention. Prophylactic methods (*e.g.*, preventing or reducing the incidence of relapse) are also considered treatment.

The amount which is administered to an individual is preferably an amount that does not induce toxic effects which outweigh the advantages which accompany its administration. For example, it is preferred that the effective amount used in the present invention does not substantively lower the treated individual's level of blood glucose. Further objectives of the present invention are to reduce in number, diminish in severity, and/or otherwise relieve suffering from the symptoms of the disease as compared to recognized standards of care. In addition to treatment of primary disease, the present invention may also be effective against metastatic disease.

A bolus administered over a short time once a day is a convenient dosing schedule. Alternatively, the effective daily dose may be divided into multiple doses for purposes of administration, for example, two to twelve doses per day. Dosage levels of active ingredients in a pharmaceutical composition can also be varied so as to achieve a transient or sustained concentration of the compound in an individual, especially in and around the tumor or other neoplasm, and to result in the desired therapeutic response. But it is also within the skill of the art to start doses of the compound at levels lower than required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved. It will be understood that the specific dose level for any particular individual will depend on a variety of factors, including body weight, general health, diet, size and change in size of the tumor or other neoplasm, route and scheduling of administration, combination with one or more other drugs, and severity of disease.

The dose level selected for use in the present invention will depend on the bioavailability, activity, and stability of the compound, the route of administration, the severity of the disease being treated, and the condition and medical history of the individual in need of treat-

ment. It is contemplated that a daily dosage may be between about one microgram to about one gram, preferably from anywhere between about 10-50 mg and about 100-500 mg (e.g., 25 to 250 mg), of the compound per kilogram body weight. Such quantities may be used in a unit dose (i.e., a dose sufficient for a single use once to several times per day). The amount
5 of compound administered is dependent upon factors known to a person skilled in this art such as, for example, the molecular weight and hydrophobicity the compound, the route of administration, location and type of tumor or other neoplasm, and the like.

In accordance with the present invention, the individual's tumor or other neoplasm may be benign or malignant. For example, the individual's disease may be classified as an
10 adenoma, carcinoma, hepatoma, or sarcoma. In particular, the bioavailability of compounds of the present invention across the blood-brain barrier makes it advantageous to treat brain cancers (e.g., astrocytomas, gliomas, meningiomas, neurinomas). Furthermore, the tumor or other neoplasm may be derived from different tissue types such as, for example, ectoderm, embryonic, endoderm, epithelium, and neuroectoderm, especially solid tissues.

15 The individual may be any animal or patient with cancer. Mammals, especially humans, may be treated by the present invention. Thus, both veterinary and medical treatments are envisioned.

The following examples are merely illustrative of the present invention and do not limit or restrict its practice.

20

EXAMPLE 1

The CT-2A brain tumor (Seyfried *et al.*, *Mol Chem Neuropathology* 17:147-167, 1992), was produced in C57BL/6J mice using the procedure of Zimmerman and Arnold (*Cancer Res* 1:919-938, 1941). Such brain tumors have been induced in mice with 20-methylcholanthrene (MC) and used extensively as animal models for evaluating chemo-
25 therapies for individuals with a brain tumor (Shaprio *et al.*, *Cancer Res* 30:2401-2413, 1970; Crafts and Wilson, *Natl Cancer Inst Monogr* 46:11-17, 1977; Zimmerman, *Ann NY Acad Sci* 381:320-324, 1982; Schold and Bigner, In: Walker, *Oncology of the Nervous System*, Martinus Nijhoff, Boston, pp. 31-64, 1983).

30 These brain tumor models are ideally suited for these studies with imino sugars and other glycosidase inhibitors because they are grown in the natural syngeneic host and have relatively simple ganglioside compositions that remain stable in both *in vitro* and *in vivo* environments. This contrasts with human glioma models where ganglioside composition changes dramatically in response to the local environment and makes it difficult to interpret

results from such models; such problems are highlighted by ganglioside analysis in a xenograft model (Ecsedy *et al.*, *J Neurochem* 73:254-259, 1999). Thus, the 20-MC brain tumor model is ideally suited for evaluating the efficacy of novel chemotherapeutics.

We have discovered that N-nonyl-1,5-deoxy-1,5-imino-D-glucitol (N-nonyl DNJ) is considerably more effective in inhibiting tumor growth than another N-alkyl-DNJ species to which it is compared. Experimental results indicate that N-nonyl DNJ is more potent than N-butyl DNJ in reducing volume and growth rate of the CT-2A tumor grown in the flanks of mice injected with CT-2A. Figure 1 shows experimental data indicating that N-nonyl DNJ inhibited experimental brain tumor growth in mice more effectively than N-butyl DNJ even when administered at one-tenth the dose (50 mg/kg/day vs. 500 mg/kg/day). These doses of N-nonyl DNJ were equivalent in effectiveness to treatment with N-butyl DNJ at a dose of 2400 mg/kg/day. Thus, the present invention using long-chain alkyl compounds, especially long-chain N-alkyl DNJ derivatives, is unexpectedly efficacious as compared to short-chain alkyl compounds. Here, a suitable dose is about 50-100 mg/kg/day.

EXAMPLE 2

Six- to eight-week old male C57BL/6J mice (Jackson Labs, Bar Harbor, ME) were inoculated subcutaneously in the flank with the CT-2A tumor line (Department of Biology, Boston College) using 0.1 cc nondissociated tumor chunks taken up to 0.2 cc with phosphate buffered saline (PBS), using an 18-gauge needle.

The following doses of N-nonyl DNJ or N-butyl DNJ, admixed with mouse chow, were administered to the control and inoculated individuals:

| | | | |
|----|-------------|--------------------|-----|
| | Control | powdered food only | n=4 |
| | N-nonyl DNJ | 50 mg/kg/day | n=3 |
| 25 | N-nonyl DNJ | 500 mg/kg/day | n=6 |
| | N-butyl DNJ | 500 mg/kg/day | n=5 |
| | N-butyl DNJ | 2400 mg/kg/day | n=2 |

Tumor volume was measured every other day beginning with day 0 of treatment, from an initial tumor volume of about 20-45 mm³, for eight days. The average \pm SEM is reported for tumor volume.

Table 1. Control mice, tumor size (mm³)

| Treatment Day | Mouse ID | | | | Average Tumor Volume |
|---------------|----------|------|------|------|----------------------|
| | #25 | #33 | #50 | #47 | |
| 0 | 27 | 30 | 35 | 38 | 33 ± 2.5 |
| 2 | 123 | 44 | 116 | 70 | 88 ± 19 |
| 4 | 281 | 135 | 214 | 255 | 221 ± 32 |
| 6 | 595 | 164 | 772 | 651 | 546 ± 132 |
| 8 | 1188 | 327 | 1566 | 1764 | 1211 ± 318 |
| Ratio 8/0 | 44.0 | 11.0 | 44.7 | 46.4 | 36.7 |

Table 2. 50 mg N-nonyl DNJ-treated mice, tumor size (mm³)

| Treatment Day | Mouse ID | | | Average Tumor Volume |
|---------------|----------|-----|------|----------------------|
| | #22 | #27 | #35 | |
| 0 | 27 | 38 | 30 | 32 ± 3.3 |
| 2 | 53 | 44 | 63 | 53 ± 5.5 |
| 4 | 89 | 125 | 111 | 108 ± 10 |
| 6 | 149 | 158 | 289 | 199 ± 45 |
| 8 | 405 | 289 | 352 | 349 ± 34 |
| Ratio: 8/0 | 15.0 | 7.6 | 11.7 | 10.9 |

5 Table 3. 500 mg N-nonyl DNJ-treated mice, tumor size (mm³)

| Treatment Day | Mouse ID | | | | | | Average Tumor Volume |
|---------------|----------|------|-----|------|------|------|----------------------|
| | #21 | #26 | #38 | #42 | #48 | #43 | |
| 0 | 38 | 40 | 23 | 36 | 23 | 30 | 32 ± 3.1 |
| 2 | 49 | 96 | 33 | 45 | 38 | 57 | 53 ± 9.3 |
| 4 | 128 | 232 | 53 | 78 | 102 | 131 | 121 ± 25 |
| 6 | 179 | 340 | 91 | 147 | 192 | 203 | 192 ± 34 |
| 8 | 357 | 442 | 150 | 378 | 384 | 430 | 357 ± 43 |
| Ratio: 8/0 | 9.4 | 11.1 | 6.5 | 10.5 | 16.7 | 13.4 | 11.1 |

Table 4. 500 mg N-butyl DNJ-treated mice, tumor size (mm³)

| Treatment Day | Mouse ID | | | | | Average Tumor Volume |
|---------------|----------|------|------|-----|------|----------------------|
| | #36 | #31 | #40 | #45 | #49 | |
| 0 | 29 | 30 | 28 | 40 | 41 | 34 ± 2.8 |
| 2 | 69 | 56 | 41 | 45 | 95 | 61 ± 10 |
| 4 | 175 | 150 | 84 | 206 | 205 | 164 ± 23 |
| 6 | 243 | 206 | 247 | 275 | 351 | 265 ± 24 |
| 8 | 616 | 540 | 306 | 381 | 547 | 478 ± 58 |
| Ratio: 8/0 | 21.2 | 18.0 | 10.9 | 9.5 | 13.3 | 14.1 |

Table 5. 2400 mg N-butyl DNJ-treated mice, tumor size (mm³)

| Treatment Day | Mouse ID | | Average Tumor Volume |
|---------------|----------|-----|----------------------|
| | #32 | #39 | |
| 0 | 42 | 48 | 45 |
| 2 | 69 | 143 | 106 |
| 4 | 195 | 149 | 172 |
| 6 | 252 | 255 | 254 |
| 8 | 346 | 332 | 339 |
| Ratio: 8/0 | 8.2 | 6.9 | 7.5 |

- 5 Reduction in tumor size during treatment compared to controls after eight days:
 - a. N-nonyl DNJ (50 mg/kg/day): 71% reduction
 - b. N-nonyl DNJ (500 mg/kg/day): 71% reduction
 - c. N-butyl DNJ (500 mg/kg/day): 61% reduction
 - d. N-butyl DNJ (2400 mg/kg/day): 72% reduction
- 10 Reduction of tumor change in size during treatment compared to controls after eight days:
 - a. N-nonyl DNJ (50 mg/kg/day): 70% reduction
 - b. N-nonyl DNJ (500 mg/kg/day): 70% reduction
 - c. N-butyl DNJ (500 mg/kg/day): 62% reduction
 - d. N-butyl DNJ (2400 mg/kg/day): 80% reduction

EXAMPLE 3

Six- to eight-week old male C57BLJ/6J mice were inoculated subcutaneously in the flank with the CT-2A tumor line using 0.1 cc nondissociated tumor chunks taken up to 0.2 cc with PBS, and an 18-gauge needle.

| | | | |
|----|-------------|------------------------------|-----|
| 5 | Control a | Powdered food only | n=3 |
| | Control b | Powdered food only, no tumor | n=3 |
| | N-nonyl DNJ | 50 mg/kg/day | n=3 |
| | N-nonyl DNJ | 500 mg/kg/day | n=3 |
| | N-butyl DNJ | 500 mg/kg/day | n=3 |
| 10 | N-butyl DNJ | 2400 mg/kg/day | n=2 |

The average \pm SEM for blood glucose concentration is reported.

Table 6. Trinder assay for blood glucose levels

| Control a (+CT-2A) | | Control b (-CT-2A) | | N-nonyl DNJ (50 mg/kg/day) | |
|--------------------|--------------|--------------------|--------------|----------------------------|--------------|
| Mouse ID | mM/L glucose | Mouse ID | mM/L glucose | Mouse ID | mM/L glucose |
| #25 | 11.5 | #1 | 12.4 | #22 | 16.7 |
| #46 | 11.0 | #2 | 15.7 | #24 | 13.6 |
| #47 | 10.7 | #3 | 11.6 | #27 | 9.9 |
| 11.0 \pm 0.2 | | 13.2 \pm 1.3 | | 13.4 \pm 2.0 | |

| N-nonyl DNJ (500 mg/kg/day) | | N-butyl DNJ (500 mg/kg/day) | | N-butyl DNJ (2400 mg/kg/day) | |
|-----------------------------|--------------|-----------------------------|--------------|------------------------------|--------------|
| Mouse ID | mM/L glucose | Mouse ID | mM/L glucose | Mouse ID | mM/L glucose |
| #21 | 12.6 | #36 | 9.7 | #28 | 6.7 |
| #37 | 11.3 | #40 | 9.5 | #32 | 6.9 |
| #48 | 9.0 | #45 | 7.4 | | |
| 11.0 \pm 1.1 | | 8.9 \pm 0.7 | | 6.8 \pm 0.1 | |

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Level of blood glucose is normal or higher with N-nonyl DNJ compared to N-butyl DNJ

- a. CT-2A-bearing controls had 16% lower blood glucose levels than non-tumor-bearing controls.
- b. N-nonyl DNJ (50 mg/kg/day)-treated mice had blood glucose levels 17% higher than CT-2A-bearing controls.
- c. N-nonyl DNJ (500 mg/kg/day)-treated mice had blood glucose levels equal to the CT-2A-bearing controls.
- d. N-butyl DNJ (500 mg/kg/day)-treated mice had blood glucose levels 19.8% lower than CT-2A-bearing controls.
- e. N-butyl DNJ (2400 mg/kg/day)-treated mice had blood glucose levels 39% lower than CT-2A-bearing controls.

EXAMPLE 4

The CT-2A tumor line was intracerebrally implanted in six- to eight-week old male C57BL/6J mice by the method of Zimmerman and Arnold (*Cancer Res* 1:919-938, 1941). Treatment with N-nonyl DNJ (330 mg/kg/day admixed into mouse chow) was initiated 48 hr post-implantation; controls were not treated. The average \pm SEM is reported for tumor dry weight after nine days of treatment.

Table 7. N-nonyl DNJ inhibits a tumor implanted in the brain

| gm Control | gm Treated |
|----------------|----------------|
| 46.7 | 11.1 |
| 51.8 | 9.0 |
| 11.2 | 22.9 |
| 27.0 | 7.3 |
| 59.2 | 16.5 |
| 16.9 | 5.8 |
| 21.9 | 6.8 |
| 14.5 | 16.2 |
| 42.2 | 9.7 |
| 31.5 | 10.1 |
| | 7.0 |
| | 7.4 |
| 32.3 \pm 5.3 | 10.8 \pm 1.5 |

This difference in tumor size is highly significant ($p < 0.001$ by two-tailed Students t test).

EXAMPLE 5

Healthy six- to eight-week old male C57BL/6J mice (*i.e.*, not tumor-bearing) were fed either N-butyl DNJ or N-nonyl DNJ (330 mg/kg/day admixed into mouse chow) for 60 days. Compound levels in various tissues were quantitated by separation using a high-performance anion-exchange column coupled with pulsed amperometric detection (HPAE-PAD) from Dionex (Sunnyvale, California, US). Measurements were made as shown in Table 8.

The uptake of N-butyl DNJ in brain and liver was much less than that of N-nonyl DNJ. Only trace quantities of N-butyl DNJ was found in the brain on days 1 and 3, and was undetectable in the brain thereafter. The quantity of N-butyl DNJ in the liver was higher than in brain, but only ranged from 0.3 $\mu\text{g/gm}$ to 1.1 $\mu\text{g/gm}$ (wet weight) when measured over the 60 days.

Table 8. Distribution of N-nonyl DNJ in various body parts

| Treatment Day | $\mu\text{g/gm}$ Wet Weight | | | |
|---------------|-----------------------------|--------|-------|-------|
| | Liver | Testes | Brain | Serum |
| 1 | 3.4 | 1.9 | 1.6 | 5.9 |
| 3 | 3.0 | 5.3 | 3.1 | 4.3 |
| 5 | 7.2 | 2.1 | 2.2 | 1.8 |
| 7 | 4.5 | 4.2 | 3.6 | 24.0 |
| 9 | 8.8 | 7.7 | 3.5 | 12.8 |
| 14 | 8.8 | 6.0 | 3.1 | 11.9 |
| 30 | 4.2 | 3.4 | 1.5 | 12.8 |
| 60 | 4.1 | 8.1 | 1.8 | 13.3 |

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To provide a possible mechanism by which the present invention may operate, but without intending to be bound by any hypothesis, the greater effectiveness of N-nonyl DNJ as compared to N-butyl DNJ in inhibiting tumor growth may be linked to its hydrophobic nature. This may result in a more favorable biodistribution for treatment of cancer, especially brain tumors.

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Furthermore, these experiments demonstrate the effect on glucose levels in the treated individuals that is associated with the impact of the N-alkyl-1,5-deoxy-1,5-imino-D-glucitol compounds. N-nonyl DNJ treated tumor-bearing individuals maintained blood glucose levels equal to or greater than that of tumor-bearing controls, while N-butyl DNJ-treated tumor-bearing individuals exhibited lower blood glucose levels. Without intending to be bound by any hypothesis, the present invention have exert a direct effect on a tumor or other neoplasm instead of simply reducing a source of energy for those cells.

N-alkyl-DNJ compounds may or may not affect tumor growth by inhibiting one or more glycolipid biosynthesis mechanisms, glucosidase-dependent cell processes, angiogenesis, and/or tumorigenesis. It is believed the effectiveness shown above is generally applicable to the treatment of all tumors dependent on the same processes to invade and expand from a local site. For that reason, the present invention is not limited to the treatment of tumors of the brain or nervous system.

All references (e.g., publications, books, patents and patent applications) cited above are indicative of the level of skill in the art and are incorporated by reference therein.

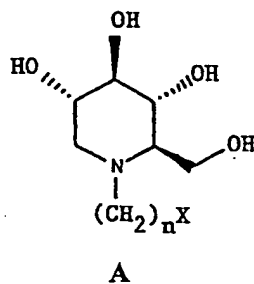
All modifications which come within the meaning of the claims and the range of their legal equivalents are to be embraced within their scope. In particular, "comprising" allows the inclusion of other elements in the claim, "comprising essentially of" allows the inclusion of other elements in the claim that do not materially affect operation of the present invention, and no particular relationship between or among elements of a claim is meant unless such limitation is explicitly recited (e.g., arrangement of components in a product claim, order of steps in a method claim).

From the foregoing, it would be apparent to a person of skill in this art that the present invention can be embodied in other specific forms without departing from its spirit or essential characteristics. The described embodiments should be considered only as illustrative, not restrictive, because the scope of the legal protection provided for the present invention will be indicated by the appended claims rather than by the foregoing description.

WE CLAIM:

1. Use of an imino sugar or pharmaceutically acceptable salt thereof to treat an individual with a tumor or other neoplasm such that growth of said tumor or other neoplasm in said treated individual is at least slowed or reduced compared to growth prior to treatment, wherein said imino sugar is not N-butyl-1,5-deoxy-1,5-imino-D-glucitol (N-butyl DNJ).
2. Use according to claim 1 wherein said imino sugar is a galactoside analog.
3. Use according to claim 1 wherein said imino sugar has an N-alkyl chain of at least five carbons.
4. Use according to claim 3 wherein said imino sugar contains at least one C₅-C₁₆ substituent.
5. Use according to claim 1 wherein bioavailability of said imino sugar across said treated individual's blood-brain barrier is substantially better than N-butyl DNJ.
6. Use according to claim 5 wherein said imino sugar is N-nonyl-1,5-deoxy-1,5-imino-D-glucitol (N-nonyl DNJ).
7. Use according to claim 1 wherein said tumor or other neoplasm is benign.
8. Use according to claim 1 wherein said tumor or other neoplasm is malignant.
9. Use according to claim 1 wherein said tumor or other neoplasm is selected from the group consisting of adenomas, carcinomas, hepatomas, and sarcomas.
10. Use according to claim 1 wherein said tumor or other neoplasm is brain cancer.
11. Use according to claim 1 wherein said brain cancer is selected from the group consisting of astrocytomas, gliomas, meningiomas, and neurinomas.
12. Use according to claim 1 wherein said tumor or other neoplasm is derived from tissue selected from the group consisting of ectoderm, embryonic, endoderm, epithelium, and neuroectoderm.

13. Use according to any one of claims 1-12 wherein treatment is administered by an enteral route.
14. Use according to any one of claims 1-12 wherein treatment is administered by a parenteral route.
15. Use according to any one of claims 1-12 wherein said individual is a mammal.
16. Use according to claim 15 wherein said mammal is a human.
17. Use according to any one of claims 1-12 wherein said treatment does not lower said individual's level of blood glucose.
18. Use of 1-deoxynojirimycin (DNJ) or a derivative thereof to treat an individual with a tumor or other neoplasm such that growth of said tumor or other neoplasm in said treated individual is at least slowed or reduced compared to growth prior to treatment, wherein said DNJ derivative is not N-butyl-1,5-deoxy-1,5-imino-D-glucitol (N-butyl DNJ).
19. Use according to claim 18 wherein a DNJ derivative according to Formula A is used,



X being selected from the group consisting of unsaturated straight aliphatic hydrocarbons, saturated and unsaturated branched aliphatic hydrocarbons, aromatic hydrocarbons and substituted derivatives thereof, cyclic hydrocarbons and substituted derivatives thereof, -O-Y, -S-Y, -Y-OH, -Y-NH₂, -Y-COOH, -Y-CON⁺-R, and -Y-COO-R;

Y being a saturated or unsaturated hydrocarbon selected from the group consisting of straight aliphatic hydrocarbons, branched aliphatic hydrocarbons, aromatic hydrocarbons and substituted derivatives thereof, and cyclic hydrocarbons and substituted derivatives thereof;

R being hydrogen or a saturated or unsaturated hydrocarbon selected from the group consisting of straight aliphatic hydrocarbons, branched aliphatic hydrocarbons, aromatic hydrocarbons and substituted derivatives thereof, cyclic hydrocarbons and substituted derivatives thereof; and

n is a whole number less than or equal to 16.

20. Use according to claim 19 wherein said derivative has an alkyl chain of at least five carbons.

21. Use according to claim 20 wherein said derivative is a C₅-C₁₆ N-alkyl derivative of DNJ.

22. Use according to claim 19 wherein bioavailability of said derivative across said treated individual's blood-brain barrier is substantially better than N-butyl DNJ.

23. Use according to claim 22 wherein said derivative is N-nonyl DNJ.

24. Use according to claim 19 wherein said tumor or other neoplasm is benign.

25. Use according to claim 19 wherein said tumor or other neoplasm is malignant.

26. Use according to claim 19 wherein said tumor or other neoplasm is selected from the group consisting of adenomas, carcinomas, hepatomas, and sarcomas.

27. Use according to claim 19 wherein said tumor or other neoplasm is brain cancer.

28. Use according to claim 27 wherein said brain cancer is selected from the group consisting of astrocytomas, gliomas, meningiomas, and neurinomas.

29. Use according to claim 19 wherein said tumor or other neoplasm is derived from tissue selected from the group consisting of ectoderm, embryonic, endoderm, epithelium, and neuroectoderm.

30. Use according to any one of claims 18-29 wherein treatment is administered by an enteral route.

31. Use according to any one of claims 18-29 wherein treatment is administered by a parenteral route.

32. Use according to any one of claims 18-29 wherein said individual is a mammal.

33. Use according to claim 32 wherein said mammal is a human.

34. Use according to any one of claims 18-29 wherein said treatment does not lower said individual's level of blood glucose.

35. Use of a glycosidase inhibitor or pharmaceutically acceptable salt thereof to treat an individual with a tumor or other neoplasm such that growth of said tumor or other neoplasm is at least slowed or reduced in said treated individual compared to growth prior to treatment, wherein said glycosidase inhibitor is not N-butyl-1,5-deoxy-1,5-imino-D-glucitol (N-butyl DNJ).

36. Use according to claim 35 wherein bioavailability of said glycosidase inhibitor across said individual's blood-brain barrier is substantially better than N-butyl DNJ.

37. Use according to any one of claims 35-36 wherein said treatment does not lower said individual's level of blood glucose.

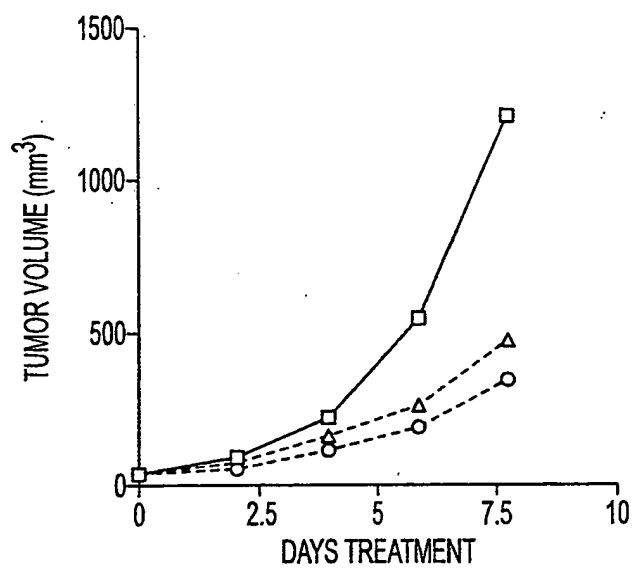


FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No

PCT/US 00/06933

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K31/445 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

CHEM ABS Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|------------|---|--------------------------|
| X,P | WO 99 24401 A (G. . SEARLE & CO.) 20 May 1999 (1999-05-20) claims 1-27 | 1-35 |
| X | EP 0 328 111 A (MEIJI SEIKA KABUSHIKI KAISHA) 16 August 1989 (1989-08-16) claim 1 | 1-5, 7-22, 24-37 |
| X | US 4 837 237 A (L. R. ROHRSCHEIDER ET AL) 6 June 1989 (1989-06-06) claims 1-13 | 1,2,5, 7-18, 30-37 |
| | — — — — — — / — | |

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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| C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT | | |
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| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
| X | PATENT ABSTRACTS OF JAPAN vol. 15, no. 92 (C-811), 6 March 1991 (1991-03-06) & JP 02 306962 A (MEIJI SEIKA KAISHA LTD), 20 December 1990 (1990-12-20) abstract | 1-5, 7-22, 24-37 |
| X | PATENT ABSTRACTS OF JAPAN vol. 16, no. 544 (C-1004), 13 November 1992 (1992-11-13) & JP 04 208264 A (TSUMURA & CO), 29 July 1992 (1992-07-29) abstract | 35-37 |
| X | PATENT ABSTRACTS OF JAPAN vol. 1998, no. 06, 30 April 1998 (1998-04-30) & JP 10 045588 A (ISHIHARA SANGYO KAISHA LTD), 17 February 1998 (1998-02-17) abstract | 35-37 |
| X | PATENT ABSTRACTS OF JAPAN vol. 1997, no. 05, 30 May 1997 (1997-05-30) & JP 09 003090 A (SANKYO CO LTD), 7 January 1997 (1997-01-07) abstract | 35-37 |
| X | PATENT ABSTRACTS OF JAPAN vol. 1996, no. 09, 30 September 1996 (1996-09-30) & JP 08 134091 A (SANKYO CO LTD), 28 May 1996 (1996-05-28) abstract | 35-37 |
| X | PATENT ABSTRACTS OF JAPAN vol. 1996, no. 07, 31 July 1996 (1996-07-31) & JP 08 059646 A (ISHIHARA SANGYO KAISHA LTD), 5 March 1996 (1996-03-05) abstract | 35-37 |
| X | PATENT ABSTRACTS OF JAPAN vol. 1995, no. 11, 26 December 1995 (1995-12-26) & JP 07 196490 A (TERUMO CORP; OTHERS: 01), 1 August 1995 (1995-08-01) abstract | 35-37 |

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No.

PCT/US 00/06933

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---------------------|----------------------------|---------------------|
| WO 9924401 A | 20-05-1999 | AU 1297399 A | 31-05-1999 |
| EP 328111 A | 16-08-1989 | JP 1207235 A | 21-08-1989 |
| | | JP 1918084 C | 07-04-1995 |
| | | JP 6043306 B | 08-06-1994 |
| | | JP 1265025 A | 23-10-1989 |
| | | JP 1991228 C | 22-11-1995 |
| | | JP 7008793 B | 01-02-1995 |
| | | JP 1268635 A | 26-10-1989 |
| | | JP 1991230 C | 22-11-1995 |
| | | JP 7008794 B | 01-02-1995 |
| | | JP 1313425 A | 18-12-1989 |
| | | JP 1313426 A | 18-12-1989 |
| | | JP 2038320 C | 28-03-1996 |
| | | JP 7068215 B | 26-07-1995 |
| | | DE 68929141 D | 24-02-2000 |
| | | US 4985445 A | 15-01-1991 |
| | | US 5250545 A | 05-10-1993 |
| US 4837237 A | 06-06-1989 | NONE | |
| JP 02306962 A | 20-12-1990 | NONE | |
| JP 04208264 A | 29-07-1992 | NONE | |
| JP 10045588 A | 17-02-1998 | NONE | |
| JP 09003090 A | 07-01-1997 | NONE | |
| JP 08134091 A | 28-05-1996 | NONE | |
| JP 08059646 A | 05-03-1996 | NONE | |
| JP 07196490 A | 01-08-1995 | NONE | |